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Cortinarius rapaceoides, a new record for Turkey

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Abstract—A macrofungus collected from Akbelen village in Tokat has been identified based on molecular evidence and morphology as *Cortinarius rapaceoides,* a new record from Turkey. Sequence data generated from the nuclear ribosomal internal transcribed spacer and ribosomal large subunit regions were deposited in GenBank and used to determine phylogenetic relationships within *Cortinarius* subg. *Phlegmacium*. A morphological description, illustrations, and phylogeny of the Turkish *C. rapaceoides* are provided.

Key words—*Cortinariaceae*, ITS, LSU, taxonomy

Introduction

The ectomycorrhizal basidiomycete genus *Cortinarius* (*Agaricales*) is the world's most speciose mushroom genus. Ectomycorrhizal associations formed with trees (e.g., *Nothofagus, Quercus, Pinus*) and herbaceous plants are important for forest ecosystem (Moser & Horak 1975, Breitenbach & Kränzlin 2000, Stensrud & al. 2014, Itoo & al. 2015). Morphological features such as cortina covering young gills, rusty brown mature gills, ochraceous-rusty to deep rusty spore print, and bulbous stipe distinguish this genus. Species separation within *Cortinarius* is greatly complicated by a high degree of inter- and intra-specific morphological variation.

Molecular markers have become important in helping resolve and refine *Cortinarius* taxonomic classification. Previous studies have used molecular markers derived from ribosomal gene loci containing the internal

transcribed spacer regions (ITS1 and ITS2), ribosomal large subunit (LSU), and the two largest subunits of RNA polymerase II (RPB1, RPB2) (Garnica & al. 2003, 2009; Froslev & al. 2005, 2007; Niskanen & al. 2009; Liimatainen & Niskanen 2013; Stensrud & al. 2014; Liimatainen & al. 2017; Sesli & Liimatainen 2018). Among those, ITS data strongly supports phylogenetic delimitation of species among *Cortinarius* sections and our understanding of the evolution of the genus (see Garnica & al. 2009). Most studies stress the necessity of combining sequence data with morphological studies to resolve the relationships among *Cortinarius* species.

Cortinarius species have been previously recorded in Turkey (Sesli & Denchev 2014; Akata & al. 2015; Güngör & al. 2015; Sesli & al. 2015, 2016; Sesli 2018; Sesli & Liimatainen 2018; Kalmer & al. 2019). Our research provides the first identification of *C. rapaceoides* in Turkey with detailed description of morphological characteristics and evidence at the molecular level.

Materials & methods

Morphological studies

Fresh *Cortinarius* basidiomes were collected from Akbelen village (Tokat) in the autumn of 2018, photographed in the field, and important morphological and ecological features noted. Basidiomes were stored in paper bags and kept in a box. The fresh collection was transported to the laboratory in paper bags, and collection numbers were assigned. The dried basidiomes were placed into polyethylene bags for storage. Tissues were mounted in Melzer's reagent, distilled water, Congo red, and KOH prior to examination under a light microscope. At least 25 measurements from each microscopic structure were obtained from a mature basidiome, which was initially identified based on its morphological features with the help of Boisselet (2012a) and Tanchaud (2016). The identified collection was deposited in the Fungarium of the Department of Biology, Tokat Gaziosmanpasa University, Tokat, Turkey (GOPUF).

Molecular studies

Genomic DNA was extracted from lamella using the ZR Fungal/Bacterial DNA MiniPrep kit as described by the manufacturer's protocol. The primer pair ITS4-ITS5 (White & al. 1990) was used to amplify ITS1-5.8S-ITS2 region and the primer pair LROR-LR5 (Vilgalys & Hester 1990) was used to amplify 28S LSU rRNA gene region. The DNA was amplified in a 30 µl volume mixture containing 3 µl 10X buffer, 3 µl dNTP mix, 3 µl degenerate primer pair (final concentration of 1 µM each), 0.3 µl Dream Taq DNA polymerase (Thermo), 10 µl gDNA and 7.7 µl sterile ddH_2O. PCR amplification for ITS region was set as: A negative control reaction using only sterile ddH_2O was also run in parallel. 5 min initial denaturation at 95°C + 40 denaturation

cycles (95°C for 30 sec) + annealing (53°C for 30 sec) + extension (72°C for 1 min) and a final extension for 10 min. The LSU PCR protocol comprised: a 3 min initial denaturation at 95°C + 40 denaturation cycles (95°C for 30 sec) + annealing (48°C for 30 sec) + extension (72°C for 1 min) and a final extension for 10 min.

PCR products were run in a 1% agarose gel electrophoresis and positive PCR products were gel purified by using Wizard SV Gel and PCR Clean-up System. Purified PCR products were sequenced in both directions using the same primer pairs by BM Labosis Inc. (Ankara).

Sequence and phylogenetic analysis

Chromatograms were checked for any nucleotide errors for each genomic sequence generated from both ends and errors were corrected manually. Assembled rDNA sequences were examined using Basic Local Alignment Search Tool (BLAST) programme to select the most closely related ITS and LSU sequences in the database. Representative ITS and LSU sequences of *Cortinarius* species were retrieved from GenBank for phylogenetic analysis. The sequence alignments and phylogenetic trees for each genomic region were analysed using MEGA 6.0 (Tamura & al. 2013). In the CLUSTALW alignment, sequences were trimmed at both ends. Phylogenetic trees were constructed using the maximum likelihood (ML) and maximum parsimony (MP) methods. Tamura-Nei model (Tamura & Nei, 1993) was used to construct the ML tree with bootstrap support of 1000 replicates and default settings. The bootstrap support values ≥50% were marked on the branches of the tree.

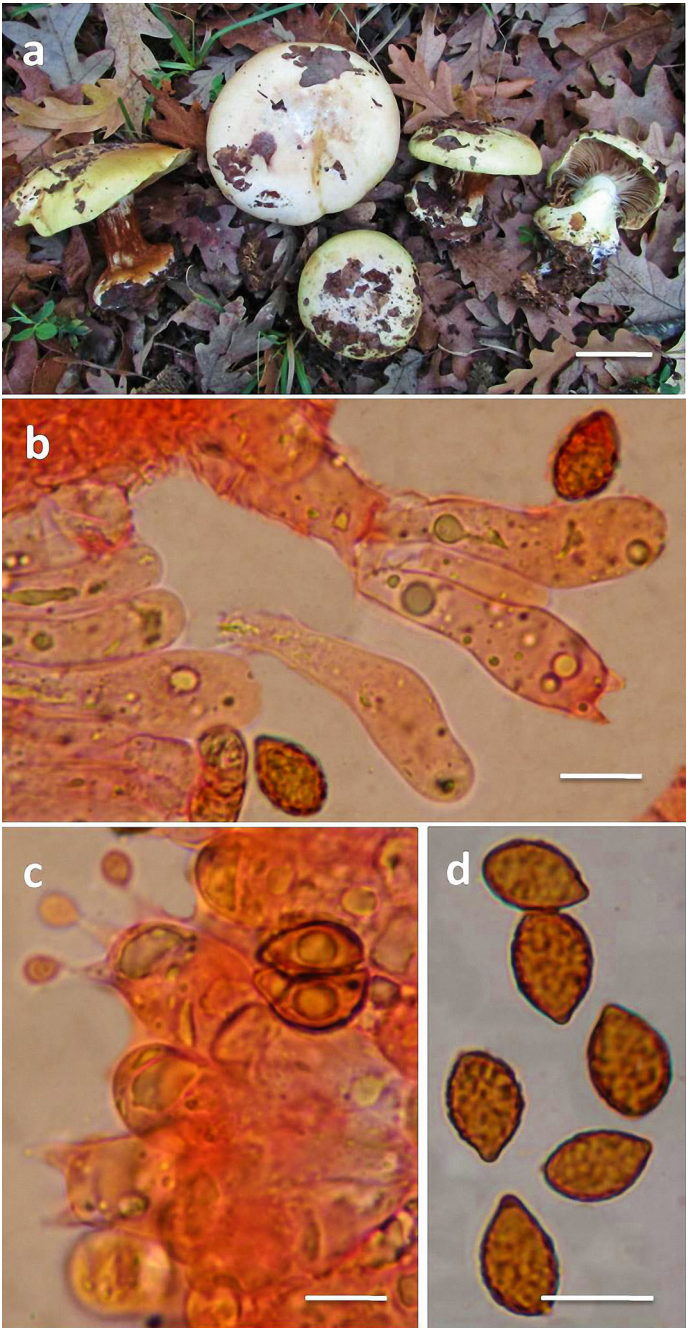
Taxonomy

Cortinarius rapaceoides Bidaud, G. Riousset & Riousset,
Micologia 2000: 68 (2000) Fig. 1

Pileus 50–100(–120) mm diam., at first convex then plane-convex to plane; surface dull when dry, lubricous when moist; colour overall yellowish to ochre-yellow, in the centre orange-yellow with darker brown squamules. Lamellae pale lilac, soon rust-brown, broad, crowded, edges smooth to slightly crenate. Stipe whitish, sometimes gray-purplish at the apex, fragile, solid, cylindrical, base with a marginate bulb, surface longitudinally whitish-fibrillose when young, brownish when old. Flesh whitish sometimes bluish in the upper and lower stipe. KOH negative on the flesh, amber yellow colour on the pileal surface. Odor of chocolate.

Basidiospores (9.6–)10–13(–13.8) × (6.0–)6.5–8(–8.5) µm, elliptical to amygdaliform, strongly verrucose, yellow-brown. Basidia, (30–)35–40(–44.6) × (9–)10.5–12(–12.4) µm, with 2–4 sterigmata, clavate.

Specimens examined: **TURKEY, Tokat, Akbelen village,** 40°27′0″N 36°39′16″E, 1050 m, among dead leaves of *Quercus* sp, 8 November 2018, leg. Isik 798 (GOPUF; GenBank MN094878, MN099705).


a
b
c
d

Habitat & distribution—Among fallen leaves on calcareous soils in deciduous woods. Previously recorded from France and Italy (Liimatainen & al. 2014).

Molecular phylogeny

The ITS1-5.8S-ITS2 (666 bp) and LSU rRNA (996 bp) sequences were deposited in GenBank. ITS sequence MN094878 BLAST results matched closely with the sequences representing *C. caroviolaceus* (EU057049), *C. rapaceoides* (KF732407, ex-holotype), and *C. saporatus* (DQ663413), which are included in the /Caroviolacei clade (Garnica & al. 2009). The remaining BLAST hits were also matched with species within the same clade. Accordingly, 67 sequences in the /Calochroi & Fulvi clade of *Cortinarius* subg. *Phlegmacium* (Liimatainen & al. 2014) were selected and ITS sequences were retrieved from GenBank for phylogenetic analyses. The outgroup *Cortinarius aureifolius* and *C. caerulescens* were used to root the phylogeny (Froslev & al. 2005, Garnica & al. 2009).

Although fewer in number, LSU sequences for genus *Cortinarius* were also selected to construct a phylogenetic tree, but the LSU-based phylogeny was not well resolved due to low bootstrap values and no phylogenetic separation at the subgenus level was observed for LSU regions.

Our two constructed MP and ML phylogenetic trees showed similar topologies for each genomic region. We present only the ML tree to indicate phylogenetic relationship of the studied species based on the ITS region. The sequence from our *Cortinarius* specimen clustered with sequences derived from *C. rapaceoides* described from Italy and France (including the ex-holotype sequence KF732407) and *C. caroviolaceus* (EU057049) from Italy with high bootstrap support (Fig. 2). Liimatainen & al. (2014) suggested that *C. caroviolaceus* sequence actually represented *C. rapaceoides* based on their ITS sequence phylogeny; and our phylogeny also supports it as conspecific with *C. rapaceoides*.

Discussion

Cortinarius rapaceoides, originally described from France and collected under *Quercus ilex* in a deciduous forest with calcareous soil, is characterized by a cream to yellow color, smooth convex pileus with a rolled margin,

Fig. 1. *Cortinarius rapaceoides* (GOPUF – Isik 798): A. basidiomata; B. basidia and basidioles; C. basidia; D. basidiospores. Scale bars: A = 3 cm; B–D = 10 μm.

cream to purple lamellae, blue, cream or purple colored cylindrical stipe with a marginate bulb, 10–13 × 6–7 µm verrucose spores, and a hardwood mycorrhizal association (Boisselet 2012a, Tanchaud 2016).

The species is morphologically similar to *C. aleuriosmus* Maire [= *C. caro-violaceus* P.D. Orton] and *C. saporatus* Britzelm. These fungi may be confused with each other due to their similar ecological features; however, *C. aleuriosmus* can be distinguished from *C. rapaceoides* by its whitish pileus and lamellae, smaller, almond-to lemon-shaped rough spores, abundant white veil, and farinaceous taste and odor (Orton 1955, Moser 1983, Phillips 1981, 2013; Boisselet 2012a, 2012b; Tanchaud 2016). *Cortinarius saporatus* differs in its bigger pileus and stipe, dark brown colour changes with KOH on the pileus, its yellowish colour at the stipe base, and an odor that is initially fruity but later more sour (Phillips 1981, 2013). Although some spores of Turkish *C. rapaceoides* are larger, the morphological characters are very similar and agree with French *C. rapaceoides* (Boisselet 2012a, Tanchaud 2016).

Sequence analyses also support identification of Turkish collection as *C. rapaceoides*. It belongs in *Cortinarius* subg. *Phlegmacium*, a polyphyletic subgenus (Peintner & al. 2004, Garnica & al. 2005). Although *Cortinarius* species are morphologically quite variable, their ITS sequences show a high sequence identity and are reliably used for species delimitation and identification (e.g. Froslev & al. 2005, 2007; Ortega & al. 2008; Garnica & al. 2009; Niskanen & al. 2009). We found the ITS region more useful than the LSU region for inferring the phylogenetic relationships and were able to determine independent phylogenetic lineages with high boostrap support. Our ITS sequence clustered in *Cortinarius* sect. *Calochroi* and showed 100% sequence identity with *C. rapaceoides* from France and Italy without any intraspecific sequence polymorphisms.

Our first record of *C. rapaceoides* in Turkey is well supported by both morphological and molecular evidence.

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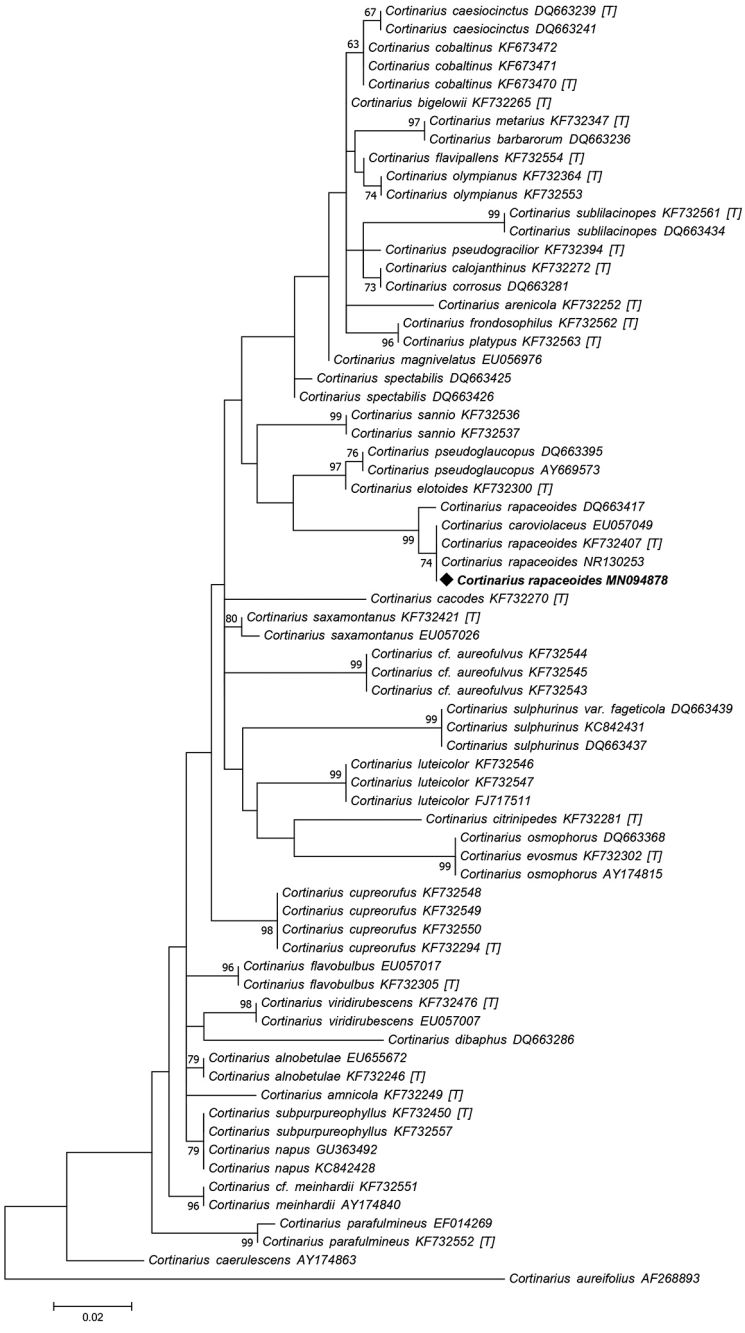
Fig. 2. Phylogeny of selected *Cortinarius* species inferred from ITS sequences using the ML method. The diamond [♦] indicates the Turkish *C. rapaceoides* identified in this study. Ex-type sequences are annotated with [T]. *Cortinarius aureifolius* and *C. caerulescens* represent the outgroup. Bootstrap support values ≥50% from ML analysis are shown on the branches. Bar = 0.02 expected changes per site per branch.


67 Cortinarius caesiocinctus DQ663239 [T]
Cortinarius caesiocinctus DQ663241
63 Cortinarius cobaltinus KF673472
Cortinarius cobaltinus KF673471
Cortinarius cobaltinus KF673470 [T]
Cortinarius bigelowii KF732265 [T]
97 Cortinarius metarius KF732347 [T]
Cortinarius barbarorum DQ663236
Cortinarius flavipallens KF732554 [T]
Cortinarius olympianus KF732364 [T]
74 Cortinarius olympianus KF732553
99 Cortinarius sublilacinopes KF732561 [T]
Cortinarius sublilacinopes DQ663434
Cortinarius pseudogracilior KF732394 [T]
Cortinarius calojanthinus KF732272 [T]
73 Cortinarius corrosus DQ663281
Cortinarius arenicola KF732252 [T]
Cortinarius frondosophilus KF732562 [T]
96 Cortinarius platypus KF732563 [T]
Cortinarius magnivelatus EU056976
Cortinarius spectabilis DQ663425
Cortinarius spectabilis DQ663426
99 Cortinarius sannio KF732536
Cortinarius sannio KF732537
76 Cortinarius pseudoglaucopus DQ663395
97 Cortinarius pseudoglaucopus AY669573
Cortinarius elotoides KF732300 [T]
Cortinarius rapaceoides DQ663417
Cortinarius caroviolaceus EU057049
99 Cortinarius rapaceoides KF732407 [T]
74 Cortinarius rapaceoides NR130253
◆ Cortinarius rapaceoides MN094878
Cortinarius cacodes KF732270 [T]
80 Cortinarius saxamontanus KF732421 [T]
Cortinarius saxamontanus EU057026
99 Cortinarius cf. aureofulvus KF732544
Cortinarius cf. aureofulvus KF732545
Cortinarius cf. aureofulvus KF732543
99 Cortinarius sulphurinus var. fageticola DQ663439
Cortinarius sulphurinus KC842431
Cortinarius sulphurinus DQ663437
99 Cortinarius luteicolor KF732546
Cortinarius luteicolor KF732547
Cortinarius luteicolor FJ717511
Cortinarius citrinipedes KF732281 [T]
Cortinarius osmophorus DQ663368
Cortinarius evosmus KF732302 [T]
99 Cortinarius osmophorus AY174815
Cortinarius cupreorufus KF732548
Cortinarius cupreorufus KF732549
98 Cortinarius cupreorufus KF732550
Cortinarius cupreorufus KF732294 [T]
96 Cortinarius flavobulbus EU057017
Cortinarius flavobulbus KF732305 [T]
98 Cortinarius viridirubescens KF732476 [T]
Cortinarius viridirubescens EU057007
Cortinarius dibaphus DQ663286
79 Cortinarius alnobetulae EU655672
Cortinarius alnobetulae KF732246 [T]
Cortinarius amnicola KF732249 [T]
Cortinarius subpurpureophyllus KF732450 [T]
Cortinarius subpurpureophyllus KF732557
79 Cortinarius napus GU363492
Cortinarius napus KC842428
Cortinarius cf. meinhardii KF732551
96 Cortinarius meinhardii AY174840
Cortinarius parafulmineus EF014269
99 Cortinarius parafulmineus KF732552 [T]
Cortinarius caerulescens AY174863
Cortinarius aureifolius AF268893
0.02

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